American Journal of Epidemiology

Vol. 153, No. 7
Printed in U.S.A.

Association of the B-Vitamins Pyridoxal 5′-Phosphate (B_6), B_{12}, and Folate with Lung Cancer Risk in Older Men

Terryl J. Hartman,[1,2] Karen Woodson,[2] Rachael Stolzenberg-Solomon,[2] Jarmo Virtamo,[3] Jacob Selhub,[4] Michael J. Barrett,[5] and Demetrius Albanes[2]

A nested case-control study was conducted within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort to test for associations between selected B-vitamins (folate, vitamin B_6, vitamin B_{12}) and incident lung cancer. This trial was conducted in Finland between 1985 and 1993. Serum was analyzed for these nutrients and homocysteine among 300 lung cancer cases and matched controls (1:1). Odds ratios and 95% confidence intervals were determined in conditional and unconditional (controlling for the matching factors) logistic regression models, after adjusting for body mass index, years of smoking, and number of cigarettes smoked per day. No significant associations were seen between serum folate, vitamin B_{12}, or homocysteine and lung cancer risk. The authors found significantly lower risk of lung cancer among men who had higher serum vitamin B_6 levels. Compared with men with the lowest vitamin B_6 concentration, men in the fifth quintile had about one half of the risk of lung cancer (odds ratio = 0.51; 95% confidence interval: 0.23, 0.93; *p*-trend = 0.02). Adjusting for any of the other serum factors (folate, B_{12}, and homocysteine) either alone or jointly did not significantly alter these estimates. This is the first report from a prospectively conducted study to suggest a role for vitamin B_6 in lung cancer. *Am J Epidemiol* 2001;153:688–94.

folic acid; homocysteine; lung neoplasms; methylation; pyridoxine; vitamin B 12

Lung cancer is a major public health problem in Western countries. In Finland, it is one of the most common cancers, with approximately 1,500 new lung cancer cases diagnosed among men each year. The age-adjusted, standardized incidence rates for 1995 were 41.6/100,000 among men (1). In the United States, the American Cancer Society estimated that there would be nearly 172,000 newly diagnosed lung cancer cases in 1999 (2).

The B-vitamins folate, B_{12}, and pyridoxal 5′-phosphate (B_6), the principal active form of vitamin B_6, are precursors of the main coenzymes involved in the transfer of one-carbon groups essential for DNA synthesis and DNA methylation. Because of their involvement in these activities, these B-vitamins have been hypothesized to be associated with carcinogenesis. The roles of folate, vitamins B_6 and B_{12}, and homocysteine in one-carbon metabolism have been reviewed in depth (3, 4). Briefly, homocysteine is thought to have two primary metabolic fates, either conversion to methionine or catabolism via the transsulfuration pathway to cystathione and eventually cysteine. These two steps are facilitated by two vitamin B_6-dependent enzymes, cystathionine beta-synthase and gamma-cystathionase. Cysteine is a main component for the synthesis of glutathione, an important cofactor of the glutathione *S*-transferases and glutathione peroxidases, which function in the detoxification of many toxic or carcinogenic compounds. Folate and vitamin B_{12} function in the methylation of homocysteine to regenerate methionine. A large proportion of methionine is activated by adenosine 5′-triphosphate to produce *S*-adenosylmethionine, the primary methyl donor to nucleic acids, neurotransmitters, phospholipids, and hormones. Methylation is well recognized as a mechanism relevant to genomic structure and function (5). Concomitant with methionine generation, tetrahydrofolate is formed that is converted to 5,10-methylenetetrahydrofolate, also facilitated by a vitamin B_6-dependent enzyme, serine hydroxymethyl transferase. 5,10-Methylenetetrahydrofolate is required for the biosynthesis of purine nucleotides and thymidylate needed for DNA synthesis and repair (5). Serum concentrations of folate, and to a lesser extent vitamins B_6 and B_{12}, are inversely correlated with serum homocysteine concentrations (6–8).

Previous studies have suggested a role for factors related to homocysteine metabolism in colon (9–13), breast (14, 15), and pancreatic (16) cancer. We examined the association between these factors and lung cancer among male smokers who participated in a large clinical trial in southwestern Finland.

Received for publication January 27, 2000, and accepted for publication September 18, 2000.
[1] Department of Nutrition, The Pennsylvania State University, University Park, PA.
[2] Division of Clinical Sciences, National Cancer Institute, Bethesda, MD.
[3] National Public Health Institute, Helsinki, Finland.
[4] US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University, Boston, MA.
[5] Information Management Services, Silver Spring, MD.
Reprint requests to Dr. Demetrius Albanes, Executive Blvd. 2120, EPS 7016, Bethesda, MD 20892 (e-mail: albanesd@mail.nih.gov).

MATERIALS AND METHODS

Sample population

We conducted a nested case-control study within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. This trial was conducted in Finland between 1985 and 1993 as a joint project between the National Public Health Institute in Finland and the US National Cancer Institute. The trial was a large, randomized, double-blind, placebo-controlled prevention trial to determine whether daily supplementation with alpha-tocopherol, beta-carotene, or both would reduce the incidence of lung or other cancers. The overall design and initial results have been published (17, 18). Briefly, 29,133 male smokers between the ages of 50 and 69 years were recruited from southwestern Finland between 1985 and 1988 and randomly assigned to one of four groups based on a 2×2 factorial design. Men who had prior cancer or serious illnesses or who reported current use of vitamins E (>20 mg/day), A (>20,000 IU/day), or beta-carotene (>6 mg/day) were ineligible. Participants received alpha-tocopherol (50 mg/day) as *dl*-alpha-tocopheryl acetate, beta-carotene (20 mg/day), both alpha-tocopherol and beta-carotene, or placebo. Active follow-up continued for 5–8 years during the trial or until April 30, 1993. Follow-up continued through the Finnish Cancer Registry and is ongoing. This clinical trial was approved by the institutional review boards of the National Cancer Institute and the National Public Health Institute of Finland.

Case ascertainment and control selection

The sample population for this analysis consisted of 300 incident lung cancer cases who were randomly selected from among those diagnosed during follow-up of the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study and who also had a whole blood sample available for DNA extraction for related studies to be conducted within the same data set. Cases were identified by the Finnish Cancer Registry (19). These were matched to 300 randomly selected controls who were cancer-free at the time of the case diagnosis on 1) age (up to ±5 years), 2) intervention group assignment, and 3) date of baseline blood collection (±28 days). The median follow-up for the group was 5.9 years.

Data collection and serum nutrient determination

At baseline, study participants completed a demographic, general medical history, and food frequency (use) questionnaire; height and weight were measured; and blood samples were collected. Information on smoking habits was collected at baseline and throughout the trial follow-up period. Data are available on the age at which smoking started, total years of smoking, number of cigarettes smoked per day, smoke inhalation (never, sometimes, often, always), and smoking cessation during active follow-up (yes/no at each visit). Serum samples were frozen at –70°C. Samples were transported on dry ice to the Vitamin Metabolism Laboratory at the Jean Mayer US Department of Agriculture Human Nutrition Research Center at Tufts University, Boston, Massachusetts, for nutrient analysis.

Serum samples were analyzed for folate, pyridoxal 5′-phosphate, vitamin B_{12}, and homocysteine. Homocysteine was determined using high performance liquid chromatography with fluorescence detection described by Araki and Sako (20). Serum folate and B_{12} were determined by radioassay using a commercial kit from Bio-Rad. Vitamin B_6 was determined by the tyrosine decarboxylase apoenzyme method of Shin-Buehring (21). Matched sets were analyzed consecutively. Blind quality control samples were labeled to resemble the subject samples and were randomly placed within each batch. The overall coefficient of variation for quality control serum nutrients was 12 percent for folate, 10 percent for pyridoxal 5′-phosphate, 6 percent for vitamin B_{12}, and 11 percent for homocysteine.

Statistical analysis

Statistical analyses were performed using Statistical Analysis System (SAS) software (22, 23). The characteristics of case subjects and control subjects were compared by the nonparametric Wilcoxon rank sum test for continuous variables and by chi-squared tests for categorical variables. Odds ratios and 95 percent confidence intervals for the association between lung cancer and serum nutrients were determined in conditional and unconditional (controlling for the matching factors) logistic regression models. Serum nutrients were analyzed as both continuous natural log-transformed variables and categorical variables. To develop the categorical variables, serum nutrient values were grouped into quintiles based on the distribution among the controls and were entered into models as indicator variables defined by the second through fifth quintiles of intake, with the lowest quintile as the referent group. To conduct a linear trend test across levels of nutrients, we created variables using exposure scores based on the median values for each quintile of serum nutrients among the controls. Dietary variables of interest, including folate, vitamins B_6 and B_{12}, methionine, alcohol, vitamin C, and fruit and vegetable intake, were also natural log transformed and in addition were adjusted for total energy intake according to the residual method of Willett and Stampfer (24). Supplemental intake of vitamins and minerals of interest was added to dietary intake before transformation and energy adjustment. A three-level smoking cessation variable was created to reflect whether the participant did not quit, quit early during follow-up (<3 years), or quit late during follow-up (≥3 years). Cessation had to be maintained for at least two consecutive follow-up visits or 8 months. Variables included in the multivariate models were those that confounded the association between serum nutrients and lung cancer and those that were risk factors for lung cancer. All regression analyses were adjusted for potential confounding by smoking (years and number of cigarettes smoked per day as continuous variables) and body mass index (weight (kg)/height $(m)^2$). Results are reported as multivariate adjusted odds ratios of lung cancer with 95 percent confidence intervals.

Effect modification by intervention, age, body mass index, smoking, alcohol intake, and other serum nutrients was assessed by including the individual factor and its cross-product term with the continuous serum nutrient variable in a separate multivariate model and by stratified analysis. For stratified analyses, variables of interest were dichotomized based on the median, and the resulting subgroups were analyzed unmatched, controlling for the matching factors and important other covariates in the models.

RESULTS

Table 1 shows selected characteristics of lung cancer cases and matched controls. The lung cancer cases were heavier smokers and had smoked for more years than the controls. In addition, the mean body mass index was significantly lower among cases compared with controls. Approximately 54 percent of the cases and controls in this study had less than adequate vitamin B_6 status (<30 nmol/liter), 90 percent had less than adequate folate status (<6 ng/ml), and only 2 percent had less than adequate vitamin B_{12} status (<190 pmol/liter). Nearly one fourth of this population had elevated serum homocysteine levels (>15 μmol/liter). These results are similar to that of a pancreatic cancer study completed in this population, with the exception that only 15 percent of pancreatic cases and controls had hyperhomocysteinemia (16). Serum concentrations of vitamins B_6, B_{12}, folate, and homocysteine were not highly correlated. The highest correlations were between serum levels of homocysteine and serum folate ($r = -0.32$) and vitamin B_{12} ($r = -0.20$). The dietary intake and serum concentrations of these B-vitamins were not highly correlated, either. The correlation between dietary intake and the serum level for vitamin B_6 was 0.23; for vitamin B_{12}, 0.10; and for folate, 0.26.

TABLE 1. Selected characteristics of lung cancer cases and controls,* Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1993

	Cases (mean (SD†))	Controls (mean (SD))
Age (years)	59.2 (4.9)	59.7 (4.4)
BMI† (kg/m^2)‡	25.5 (3.6)	26.3 (3.6)
Smoking		
Cigarettes/day‡	22.2 (9.0)	20.2 (9.5)
Years smoking‡	40.6 (6.7)	37.8 (8.0)
Dietary intake		
Energy (kcal)	2,748 (764)	2,790 (808)
Alcohol (g)	16.3 (20.8)	16.4 (21.1)
Methionine (mg)	1,996 (633)	2,056 (597)
Folate (μg)	320 (100)	331 (100)
Folate (μg)§	333 (114)	341 (105)
Vitamin B_{12} (μg)	10.8 (5.0)	11.0 (4.5)
Vitamin B_{12} (μg)§	15.5 (39.7)	12.9 (24.3)
Vitamin B_6 (mg)	2.4 (0.7)	2.5 (0.8)
Vitamin B_6 (mg)§	7.8 (44.2)	6.2 (38.0)
Serum		
Folic acid (ng/ml)	4.3 (1.9)	4.3 (1.8)
Vitamin B_{12} (pg/ml)	479 (159)	460 (152)
Vitamin B_6 (nmol/liter)‡	43.3 (71.0)	45.6 (59.4)
Homocysteine (μmol/liter)	12.8 (4.4)	13.4 (7.0)

* Number of cases, 300; number of controls, 300.
† SD, standard deviation; BMI, body mass index (weight (kg)/height (m)^2).
‡ Cases significantly different ($p < 0.05$) from controls by Wilcoxon's test.
§ Including supplements.

Table 2 presents results from multivariate conditional regression models. After controlling for body mass index, years of smoking, and the number of cigarettes smoked per day, we found significantly lower risk of lung cancer among men who had higher serum vitamin B_6 concentrations. Compared with men having the lowest serum B_6 concentrations, men in both the fourth and fifth quintiles had about one half of the risk of lung cancer (quintile 4 odds ratio = 0.46; 95 percent confidence interval: 0.25, 0.83; quintile 5 odds ratio = 0.51; 95 percent confidence interval: 0.23, 0.93; p-trend = 0.02). Adjusting for the other serum factors (folate, B_{12}, and homocysteine) either alone or jointly did not significantly alter these estimates. In addition, adjusting for dietary variables (e.g., fruit and vegetables, vitamin C, or total energy intake) or other smoking variables (year at which started smoking, smoke inhalation patterns, and smoking cessation during follow-up) did not significantly alter these estimates. We did not see any associations between levels of vitamin B_{12}, folate, or homocysteine with lung cancer risk after controlling for vitamin B_6 and other covariates. Dietary vitamin B_6, vitamin B_{12}, and folate were only weakly associated with lung cancer, and a combination of low folate intake, low methionine intake, and high alcohol intake was not associated with lung cancer risk (data not shown).

There were no significant interactions between serum vitamin B_6 and serum folate level, alcohol intake, age, body mass index, or treatment group. The risk estimates for quintiles of serum vitamin B_6 stratified by factors known to affect methyl group availability (smoking, methionine and alcohol intake, and serum homocysteine, folate, and vitamin B_{12}) are shown in table 3. There was a statistically significant interaction between vitamin B_6 and smoking, with the protective effect of vitamin B_6 apparent only among those who had smoked longer. There was a statistically significant interaction between dietary methionine intake and serum vitamin B_6, with a consistent pattern of decreasing risk of lung cancer with increasing serum vitamin B_6 concentration apparent only among those with lower methionine intakes. There was also a statistically significant interaction between serum vitamin B_6 and serum vitamin B_{12}. This association, however, did not show a consistent dose-response pattern in either stratum of vitamin B_{12}. There was a marginally significant interaction between serum vitamin B_6 and serum homocysteine ($p = 0.05$), with the suggestion that the protective effect of vitamin B_6 was stronger among men with higher homocysteine levels. In addition, although we observed no main effects for serum homocysteine con-

TABLE 2. Adjusted odds ratios and 95% confidence intervals of lung cancer by baseline serum nutrient quintiles, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1993

Serum nutrient and quintile	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval	*p*-trend
Vitamin B_6 (nmol/liter)*					
1 (≤20.6)	86	59	1.0		
2 (20.7–26.1)	69	59	0.80	0.47, 1.35	
3 (26.2–35.0)	59	58	0.61	0.34, 1.08	
4 (35.1–48.2)	37	59	0.46	0.25, 0.83	
5 (>48.2)	52	58	0.51	0.28, 0.93	0.02
Vitamin B_{12} (pg/ml)†					
1 (≤345)	60	58	1.0		
2 (346–407)	45	58	0.82	0.46, 1.47	
3 (408–474)	55	59	0.98	0.57, 1.68	
4 (475–580)	65	58	1.13	0.64, 1.97	
5 (>580)	71	58	1.41	0.80, 2.50	0.14
Folate (μg/ml)†					
1 (≤3.1)	69	60	1.0		
2 (3.2–3.6)	35	58	0.37	0.19, 0.72	
3 (3.7–4.2)	60	58	0.84	0.47, 1.51	
4 (4.3–5.2)	75	63	1.17	0.66, 2.10	
5 (>5.2)	54	55	0.96	0.52, 1.79	0.28
Homocysteine (μmol/liter)†					
1 (≤9.6)	71	59	1.0		
2 (9.7–11.3)	49	59	0.70	0.40, 1.24	
3 (11.4–12.7)	48	58	0.75	0.43, 1.30	
4 (12.8–15.7)	72	59	1.04	0.59, 1.82	
5 (>15.7)	54	58	0.61	0.32, 1.17	0.41

* Adjusted for body mass index, smoking (years), and cigarettes per day.
† Also adjusted for vitamin B_6.

centrations, among men with high serum vitamin B_6 levels, serum homocysteine was significantly inversely associated with lung cancer risk with a significant trend ($p = 0.007$).

DISCUSSION

In this nested case-control study of lung cancer, we found an inverse association between the serum vitamin B_6 concentration and risk. Serum vitamin B_{12}, folate, and homocysteine were not associated with lung cancer incidence.

Very few studies have evaluated the association between serum vitamin B_6, vitamin B_{12}, folate, or homocysteine and lung cancer. To our knowledge, no other study has evaluated these serum factors jointly in relation to lung cancer. Bandera et al. (25) reported an inverse association between dietary folate and lung cancer among men but not among women in the New York State cohort, and another case-control study found a nonsignificant inverse trend for folate intake and lung cancer (26). In a case-control study conducted in Turkey, plasma B_{12} levels were higher among lung cancer cases compared with controls, while levels of serum folate in those with lung cancer did not differ significantly from those of controls (27). Heimburger et al. (28) evaluated whether folate and vitamin B_{12} supplementation modified the severity of potentially premalignant lesions among 73 men with a history of 20 or more pack-years of cigarette smoking. Men who had taken supplements with folate and vitamin B_{12} showed significantly greater reduction of atypia compared with men who received a placebo. This group also reported that plasma folate levels were lower in smokers with lung metaplasia than in smokers without metaplasia (28).

The finding of an inverse association between serum vitamin B_6 and lung cancer is intriguing. There are several potential mechanisms by which vitamin B_6 may influence carcinogenesis. Vitamin B_6 is known to affect immunocompetence (29–33). Some animal research also demonstrates that vitamin B_6 deficiency leads to greater lipid peroxidation in plasma and liver when the animal consumes a high fat diet (34, 35). Vitamin B_6 also plays an important role in homocysteine metabolism serving a critical role in homocysteine catabolism. In the process of homocysteine catabolism, cysteine, a main component for the synthesis of glutathione, is generated. Glutathione serves as an important cofactor of the glutathione *S*-transferases and glutathione peroxidases, which function in the detoxification of many toxic or carcinogenic compounds (5, 36). Decreased activity of these enzymes has been associated with increased DNA

TABLE 3. Adjusted odds ratios and 95% confidence intervals of lung cancer by strata of selected baseline characteristics, Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study cohort, 1985–1993

Serum B_6 quintile*	Low smoking (≤40 years)				High smoking (>40 years)†			
	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval
1	29	35	1.0		57	24	1.0	
2	30	43	0.67	0.32, 1.39	30	16	0.81	0.36, 1.84
3	33	40	0.84	0.41, 1.74	26	18	0.58	0.26, 1.33
4	20	36	0.50	0.23, 1.13	17	28	0.28	0.12, 0.66
5	30	31	1.12	0.52, 2.40	22	27	0.32	0.14, 0.69
				p-trend = 0.45				*p*-trend = 0.002
	Low alcohol (≤9 g/day)				High alcohol (>9 g/day)†			
	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval
1	50	34	1.0		26	23	1.0	
2	31	30	0.75	0.37, 1.53	26	26	0.92	0.39, 2.13
3	24	30	0.65	0.31, 1.35	35	26	1.10	0.48, 2.52
4	12	22	0.47	0.20, 1.14	22	35	0.53	0.23, 1.22
5	24	26	0.68	0.32, 1.48	27	30	0.83	0.37, 1.90
				p-trend = 0.36				*p*-trend = 0.58
	Low methionine (≤1,990 mg/day)				High methionine (>1,990 mg/day)†			
	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval
1	48	26	1.0		28	31	1.0	
2	37	26	0.63	0.29, 1.36	20	30	0.75	0.33, 1.69
3	24	28	0.61	0.27, 1.38	35	28	1.29	1.60, 2.78
4	20	26	0.45	0.19, 1.06	14	31	0.41	0.17, 0.98
5	22	34	0.23	0.10, 0.53	29	22	1.61	0.71, 3.66
				p-trend = 0.0005				*p*-trend = 0.23
	Low serum B_{12} (≤451.7 pg/ml)				High serum B_{12} (>451.7 pg/ml)†			
	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval
1	48	35	1.0		38	24	1.0	
2	32	39	0.56	0.28, 1.12	28	20	1.15	0.51, 2.62
3	25	23	0.84	0.39, 1.82	34	35	0.71	0.34, 1.49
4	22	16	1.09	0.47, 2.55	15	43	0.22	0.10, 0.52
5	15	32	0.30	0.13, 0.68	37	26	1.10	0.51, 2.37
				p-trend = 0.01				*p*-trend = 0.86

Table continues

damage in lung cancer tissue (37). Vitamin B_6 may affect DNA synthesis through its intricate involvement with folic acid. Vitamin B_6 facilitates the transfer of a methyl group to tetrahydrofolate, yielding 5,10-methylenetetrahydrofolate needed for reactions generating thymidylate and purines. Thus, lower serum B_6 levels might lead to reduced DNA synthesis and impaired DNA repair (5, 36). Further, disruption of these reactions may lead to imbalances in methyl groups required for methylation processes, including DNA methylation. Altered DNA methylation has been observed in tumors at several sites (36, 38).

We observed that several other factors related to methyl group availability modified the protective effect observed for increasing serum vitamin B_6 status and lung cancer. For dietary methionine, serum vitamin B_{12}, and serum homocysteine, the protective effects of vitamin B_6 were more apparent in the strata more likely to be susceptible to imbalances in methyl group availability for use in DNA methylation or synthesis (higher homocysteine, lower methionine, lower vitamin B_{12}). It is unclear why the protective effect of vitamin B_6 was more consistent among those who had smoked longer; however, cigarette smoking has been

TABLE 3. Continued

Serum B_6 quintile*	Low serum folate (≤3.9 μg/ml)				High serum folate (>3.9 μg/ml)†			
	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval
1	52	37	1.0		34	22	1.0	
2	28	31	0.72	0.36, 1.44	32	28	0.71	0.33, 1.57
3	21	28	0.56	0.26, 1.20	38	30	0.85	0.39, 1.85
4	11	27	0.29	0.12, 0.68	26	32	0.57	0.26, 1.27
5	18	23	0.54	0.25, 1.19	34	35	0.64	0.30, 1.39
				p-trend = 0.09				*p*-trend = 0.33
	Low serum homocysteine (≤11.9 μm/liter)				High serum homocysteine (>11.9 μm/liter)†			
	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval	Cases (no.)	Controls (no.)	Odds ratio	95% confidence interval
1	31	28	1.0		55	31	1.0	
2	28	34	0.60	0.27, 1.33	32	25	0.81	0.39, 1.67
3	32	29	0.95	0.42, 2.14	27	29	0.54	0.26, 1.10
4	24	24	1.04	0.44, 2.45	13	35	0.19	0.08, 0.42
5	25	29	0.71	0.31, 1.61	27	29	0.50	0.24, 1.03
				p-trend = 0.70				*p*-trend = 0.05

* All models are adjusted for body mass index, smoking (years), cigarettes per day, season of blood draw, age, and trial treatment.
† *p*-interaction = 0.03 (smoking), 0.69 (alcohol), 0.04 (methionine), 0.004 (serum B_{12}), 0.83 (serum folate), and 0.05 (serum homocysteine).

reported to affect vitamin B_6 status and is also known to interfere with folate and vitamin B_{12} metabolism (39–41). The number of effect modifiers evaluated among these data dictate caution in interpreting these findings; however, these results appear internally consistent.

We did not see any strong associations between dietary intake of B-vitamins and lung cancer in this study. The correlations between diet and serum markers in this population were somewhat lower than those observed by others. Willett et al. (42) reported correlations of 0.37 for both vitamin B_6 and vitamin B_{12}. Jacques et al. (43) and Selhub et al. (44) reported correlations of 0.63 and 0.56, respectively, for folate. As noted above, smoking and alcohol are known to affect B-vitamin status and may have interfered with the metabolism of these nutrients in this population.

The prospective design of this study minimizes the potential for recall bias and the effects of disease on serum micronutrient measurements. Moreover, by measuring all three of the nutrients known to be important to homocysteine metabolism, we were able to adjust for their potential confounding and contribution to DNA synthesis, repair, or methylation via these pathways. Some issues should be considered when weighing the results of our study. These results may not be readily generalizable to other populations. Many men in this population had inadequate levels of folate and vitamin B_6. All of the men in this population are smokers. Both smoking and alcohol have been inversely associated with both nutrients (45–48). It is also possible that we may not have captured the right time period to see an effect for the serum vitamins other than vitamin B_6. Other studies should shed more light on this possibility. Low vitamin B_6 status may occur as a result of early, as yet undiagnosed lung cancer. While it is feasible that poorer vitamin B_6 status reflects subclinical disease, these results were unchanged when persons with cancers diagnosed during the first 2 years of follow-up were eliminated from the analysis. Theophylline, prescribed for lung disorders such as chronic bronchitis, asthma, and emphysema, is known to lower serum vitamin B_6 concentrations (49, 50). These three conditions have also been positively associated with lung cancer risk (51). In these analyses, inclusion of a variable combining these lung conditions in the final models did not appreciably alter the risk estimates for vitamin B_6.

In conclusion, our findings suggest an inverse association between vitamin B_6 and risk of lung cancer among older male smokers. We did not find evidence for a protective association between higher concentrations of serum folate or vitamin B_{12} and decreased lung cancer. This is the first report of a protective effect of vitamin B_6 for lung cancer in a prospective study; therefore, the findings should be replicated in other populations.

REFERENCES

1. Finnish Cancer Registry-Institute for Statistical and Epidemiological Cancer Research. Cancer incidence in Finland, 1995. In: Cancer statistics of the National Research and Development Centre for Welfare and Health. Helsinki, Finland: Cancer Society of Finland, 1997. (Publication no. 58).
2. Landis SH, Murray T, Bolden S, et al. Cancer statistics, 1999. CA Cancer J Clin 1999;49:8–31.
3. Bailey LB, Gregory JF 3rd. Folate metabolism and requirements. J Nutr 1999;129:779–82.

4. Selhub J, Miller JW. The pathogenesis of homocysteinemia: interruption of the coordinate regulation by *S*-adenosylmethionine of the remethylation and transsulfuration of homocysteine. Am J Clin Nutr 1991;55:131–8.
5. Eto I, Krumdieck CL. Role of vitamin B_{12} and folate deficiencies in carcinogenesis. In: Poirer LA, Newbune PM, Poriza MW, eds. Essential nutrients in carcinogenesis. New York, NY: Plenum Press, 1985:313–30.
6. Selhub J, Jacques PF, Wilson PW, et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
7. Stabler SP, Marcell PD, Podell ER. Elevation of total homocysteine in the serum of patients with cobalamin or folate deficiency detected by capillary gas chromatography-mass spectrometry. J Clin Invest 1988;81:466–74.
8. Ubbink JB, van der Merwe A, Delport R, et al. The effect of a subnormal vitamin B_6 status on homocysteine metabolism. J Clin Invest 1996;98:177–84.
9. Baron JA, Sandler RS, Haile RW, et al. Folate intake, alcohol consumption, cigarette smoking, and risk of colorectal adenomas. J Natl Cancer Inst 1998;90:57–62.
10. Chen J, Giovannucci EL, Hunter DJ. MTHFR polymorphism, methyl-replete diets and the risk of colorectal carcinoma and adenoma among U.S. men and women: an example of gene-environment interactions in colorectal tumorigenesis. J Nutr 1999;129(suppl):560S–4S.
11. Giovannucci E, Rimm EB, Ascherio A, et al. Alcohol, low-methionine–low-folate diets, and risk of colon cancer in men. J Natl Cancer Inst 1995;87:265–73.
12. Glynn SA, Albanes D, Pietinen P, et al. Colorectal cancer and folate status: a nested case-control study among male smokers. Cancer Epidemiol Biomarkers Prev 1996;5:487–94.
13. Kato I, Dnistrian AM, Schwartz M, et al. Serum folate, homocysteine and colorectal cancer risk in women: a nested case-control study. Br J Cancer 1999;79:1917–22.
14. Wu K, Helzlsouer KJ, Comstock GW, et al. A prospective study on folate B_{12} and pyridoxal 5′-phosphate (B_6) and breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:209–17.
15. Zhang S, Hunter DJ, Hankinson SE, et al. A prospective study of folate intake and the risk of breast cancer. JAMA 1999; 281:1632–7.
16. Stolzenberg-Solomon RZ, Albanes D, Nieto FJ, et al. Pancreatic cancer risk and nutrition-related methyl-group availability indicators in male smokers. J Natl Cancer Inst 1999;91:535–41.
17. The ATBC Study Cancer Prevention Study Group. The effect of vitamin E and beta-carotene on the incidence of lung and other cancers in male smokers. N Engl J Med 1994;330:1029–35.
18. The ATBC Study Cancer Prevention Study Group. The Alpha-Tocopherol, Beta-Carotene Lung Cancer Prevention Study: design, methods, participant characteristics, and compliance. Ann Epidemiol 1994;4:1–10.
19. Kyllonen LE, Teppo L, Lehtonen M. Completeness and accuracy of registration of colorectal cancer in Finland. Ann Chir Gynaecol 1987;76:185–90.
20. Araki A, Sako Y. Determination of free and total homocysteine in human plasma by high-performance liquid chromatography with fluorescence detection. J Chromatogr 1987;422:43–52.
21. Shin-Buehring Y, Rasshofer R, Endres W. A new enzymatic method for pyridoxal-5′-phosphate determination. J Inherit Metab Disorders 1981;4:123–4.
22. SAS Institute, Inc. SAS/STAT software changes and enhancements through release 6.11: the PHREG procedure. Cary, NC: SAS Institute, Inc, 1991.
23. SAS Institute, Inc. SAS/STAT user's guide, version 6. 4th ed. Cary, NC: SAS Institute, Inc, 1994.
24. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
25. Bandera EV, Freudenheim JL, Marshall JR, et al. Diet and alcohol consumption and lung cancer risk in the New York State cohort. Cancer Causes Control 1997;8:828–40.
26. Le Marchand L, Yoshizawa CN, Kikibek KB, et al. Vegetable consumption and lung cancer risk: a population-based case-control study in Hawaii. J Natl Cancer Inst 1989;81:1158–64.
27. Gurdal-Yuksel E, Karadag M, Ozyardimci N, et al. Cigarette smoking, serum lipids, folate, and vitamin B_{12} in lung cancer. J Environ Pathol Toxicol Oncol 1996;15:161–7.
28. Heimburger DC, Alexander B, Birch R, et al. Improvement in bronchial squamous metaplasia in smokers treated with folate and vitamin B_{12}. JAMA 1988;259:1525–30.
29. Miller LT, Kerkvliet NI. Effect of vitamin B_6 on immunocompetence in the elderly. Ann N Y Acad Sci 1990;587:49–54.
30. Talbott MC. Pyridoxine supplementation: effect on lymphocyte responses in elderly persons. Am J Clin Nutr 1987;46:659–64.
31. Doke S, Inagaki N, Hayakawa T, et al. Effect of vitamin B_6 deficiency on an antibody production in mice. Biosci Biotechnol Biochem 1997;61:1331–6.
32. Rall LC, Meydani SN. Vitamin B_6 and immune competence. Nutr Rev 1993;51:217–25.
33. Meydani SN, Ribaya-Mercado JD, Russell RM, et al. Vitamin B-6 deficiency impairs interleukin 2 production and lymphocyte proliferation in elderly adults. Am J Clin Nutr 1991;53:1275–80.
34. Ravichandran V, Selvam R. Increased lipid peroxidation by vitamin B-6 deficient rats. Biochem Int 1990;21:599–605.
35. Selvam R, Ravichandran V. Effect of oral methionine and vitamin E on blood lipid peroxidation in vitamin B_6 deficient rats. Biochem Int 1991;23:10007–17.
36. Jarrard DF, Bova GS, Isaacs WB. DNA methylation, molecular genetic, and linkage studies in prostate cancer. Prostate Suppl 1996;6:36–44.
37. Jaruga P. Oxidative DNA base damage and antioxidant enzyme activities in human lung cancer. FEBS Lett 1994;341:59–64.
38. Jones PA, Buckley JD. The role of DNA methylation in cancer. Adv Cancer Res 1990;54:1–23.
39. Giraud DW, Martin HD, Driskell JA. Erythrocyte and plasma B-6 vitamin concentrations of long-term tobacco smokers, chewers, and nonusers. Am J Clin Nutr 1995;62:104–9.
40. Piyathilake CJ, Macaluso M, Hine RJ, et al. Local and systemic effects of cigarette smoking on folate and vitamin B-12. Am J Clin Nutr 1994;60:559–66.
41. Dalery K, Lussier-Cacan S, Selhub J, et al. Homocysteine and coronary artery disease in French Canadian subjects: relation with vitamins B_{12}, B_6, pyridoxal phosphate, and folate. Am J Cardiol 1995;75:1107–11.
42. Willett WC, Stampfer MJ, Underwood BA, et al. Validation of a dietary questionnaire with plasma carotenoid and alpha-tocopherol levels. Am J Clin Nutr 1983;38:631–9.
43. Jacques PF, Sulsky SI, Sadowski JA. Comparison of micronutrient intake measured by a dietary questionnaire and biochemical indicators of micronutrient status. Am J Clin Nutr 1993;57: 182–9.
44. Selhub J, Jacques PF, Wilson PW, et al. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693–8.
45. Bitsch R. Vitamin B_6. Int J Vitam Nutr Res 1993;63:278–82.
46. Feinman L, Filer LJ. Nutrition and diet in alcoholism. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia, PA: Lea & Febiger, 1994:1081–97.
47. Laklem JE. Vitamin B_6. In: Shils ME, Olson JA, Shike M, eds. Modern nutrition in health and disease. 8th ed. Philadelphia, PA: Lea & Febiger, 1994:383–401.
48. Herbert V. Vitamin B_{12}. In: Ziegler EE, Filer LJ Jr, eds. Present knowledge in nutrition. 7th ed. Washington, DC: International Life Sciences Foundation, 1996:191–205.
49. Ubbink JB, Delport R, Becker PJ, et al. Evidence of a theophylline-induced vitamin B_6 deficiency caused by noncompetitive inhibition of pyridoxal kinase. J Lab Clin Med 1989;113: 15–22.
50. Ubbink JB, Delport R, Bissbort S, et al. Relationship between vitamin B-6 status and elevated pyridoxal kinase. J Nutr 1990; 120:1352–9.
51. Mayne ST, Buenconsejo J, Janerich DT. Previous lung disease and risk of lung cancer among men and women nonsmokers. Am J Epidemiol 1999;149:13–20.